Influence of extra- and intra-oral application of CPP-ACP and fluoride on re-hardening of eroded enamel

Wegehaupt, F J ; Tauböck, T T ; Stillhard, A ; Schmidlin, P R ; Attin, T

Abstract: Abstract Objectives. This in-situ study aimed to investigate the potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) crème and fluoride mouth rinse to re-harden erosively softened enamel and to evaluate the influence of an intra-oral or extra-oral application. Methods. Ten volunteers performed five experimental series. Per series, four bovine enamel samples were extra-orally softened by immersion in Sprite light (2 min) and subsequently worn intra-orally for 5 min in intra-oral appliances. Thereafter, samples were treated (3 min) with either 250 ppm AmF/SnF(2) solution (Meridol) (series 1 and 3) or CPP-ACP crème (Tooth Mousse) (series 2 and 4). Application of the substances was performed extra-orally (series 1 and 2) or intra-orally (series 3 and 4). Untreated specimens served as control (series 5). The appliances were worn for 4 h afterwards. Knoop microhardness (KHN) measurement was performed at baseline, after softening and after completing of the respective run. Data were statistically analyzed by ANOVA and Bonferroni/Dunn post-hoc test. Results. No significant difference in baseline microhardness was observed, while immersion in Sprite light reduced the microhardness significantly. Significant re-hardening after intra-oral exposure occurred in all series, but baseline microhardness was not achieved. Microhardness in series 2 was significantly higher than that in series 1 and 5. No significant differences in KHN were detected between series 3, 4 and 5. The re-hardening ΔKHN (final microhardness - microhardness after erosion) was not significant different in all five series. Conclusion. Intra-oral application of CPP-ACP crème or fluoride solution provides no benefit regarding re-hardening of erosively softened enamel.

DOI: https://doi.org/10.3109/00016357.2011.600713

Influence of extra- and intraoral application of CPP-ACP and fluoride on re-hardening of eroded enamel.

FLORIAN J. WEGEHAUPT*[1], TOBIAS T. TAUBÖCK[1], ANGELA STILLHARD[1], PATRICK R. SCHMIDLIN[1] & THOMAS ATTIN[1]

[1]Clinic for Preventive Dentistry, Periodontology and Cariology University of Zurich, Plattenstrasse 11, CH-8032 Zürich (Switzerland)

*Corresponding author:

Dr. Florian Wegehaupt

Clinic for Preventive Dentistry, Periodontology and Cariology, University of Zurich

Plattenstrasse 11

CH-8032 Zürich (Switzerland)

Tel. +41 44 634 3354, Fax. +41 44 634 4308,

E-Mail: florian.wegehaupt@zzm.uzh.ch

Short title: Enamel rehardening by CPP-ACP crème.

Word count abstract: 248

Figures: 1

Tables: 1

Abstract:

Objectives: This *in-situ*-study aimed to investigate the potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) crème and fluoride mouth rinse to re-harden erosivly softened enamel and to evaluate the influence of an intraoral or extraoral application.

Methods: Ten volunteers performed five experimental series. Per series, four bovine enamel samples were extraorally softened by immersion in Sprite light® (2 min) and subsequently worn intraorally for 5 min in intraoral appliances. Thereafter, samples were treated (3 min) with either 250 ppm AmF/SnF_2 solution (Meridol) (series 1 and 3) or CPP-ACP crème (Tooth Mousse) (series 2 and 4). Application of the substances was performed extraorally (series 1 and 2) or intraorally (series 3 and 4). Untreated specimens served as control (series 5). The appliances were worn for four hours afterwards. Knoop microhardness (KHN) measurement was performed at baseline, after softening and after completing of the respective run. Data were statistically analyzed by ANOVA and Bonferroni/Dunn post-hoc test.

Results: No significant difference in baseline microhardness was observed, while immersion in Sprite light reduced the microhardness significantly. Significant re-hardening after intraoral exposure occurred in all series, but baseline microhardness was not achieved. Microhardness in series 2 was significantly higher than that in series 1 and 5. No significant differences in KHN were detected between series 3, 4 and 5, respectively.

The re-hardening ΔKHN (final microhardness – microhardness after erosion) was not significant different in all five series.

Conclusion: Intraoral application of CPP-ACP crème or fluoride solution provides no benefit regarding re-hardening of erosivly softened enamel.

Key words

erosion, in situ study, casein phosphopeptide-amorphous calcium phosphate

Introduction:

Over the last decades dental hard tissues loss due to caries has declined [1]. However, other causes of dental hard tissue loss or disintegration have entered into the focus of dentistry, e.g. erosion and abrasion [2,3]. Dental erosion is defined as surface dissolution of dental hard tissues due to chemical processes not involving bacteria [4]. The main etiological factors, beside chelators, are acids of intrinsic)[5] (e.g. gastric acid) or extrinsic [6] (e.g. dietarian) origin. Beside the bulk tissue loss by the chemical dissolution of dental hard tissues, erosions are accompanied by the softening of the superficial dental hard tissues due to partial demineralisation, leading to a higher susceptibility due to abrasion or attrition [7,8]. In the oral cavity, dental hard tissues softened by the contact with acids are remineralised by minerals provided by the saliva [9]. With increasing remineralisations time, the susceptibility of softened enamel due to abrasion decreases, but even after 60 min of saliva exposure softened enamel had a significant higher susceptibility due to abrasion compared with unsoftened enamel [10]. To prevent the erosive and erosive/abrasive tooth wear, different approaches on the basis of fluoride application on the sound enamel to increase the resistance against demineralisation [11,12] or on the demineralised enamel to decrease the susceptibility due to abrasion by remineralisation [13,14] have been established and discussed in the literature [3,15,16]. As the remineralisation of previously eroded enamel by saliva occur due to the disposition of minerals like calcium and phosphate, it might be conceivable and has been reported in literature, that this remineralisation could although occur by rinsing the mouth with milk [17,18]. From a theoretical consideration it might be assumed that this remineralisation might be more effective than that caused by saliva due to the higher concentration of calcium and phosphate in milk. However, this assumption could not be certified by a study of Wiegand et al. (2008) [18], as no statistically significant higher re-hardening of previously softened enamel due to milk rinsing compared with saliva

contact only was observed. Also, the re-hardening after rinsing with milk was less pronounced compared with the rinsing with a fluoride containing mouth rinse.

A resent study [19] showed protective effects of casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) containing crème against erosive/abrasive tooth wear. Furthermore, Tantbirojn et al. (2008) [20] reported a significantly hardening of enamel softened by a cola drink due to the application of CPP-ACP paste. ACP is biologically active and is able to release calcium and phosphate to keep up the saturation within the fluid phase surrounding the tooth structures, thus enhancing the remineralization process [20] while the CPP is able to stabilise calcium phosphate in solution and substantially increase the level of calcium phosphate [21]. Previous studies have shown that CPP–ACP is able to enhance the remineralization of carious lesions [22,23]. Beside the direct remineralisation effect of CPP-ACP due to the high concentration of calcium and phosphate also an interaction with the saliva, such as stimulation of the salivary flow rate or interaction with certain salivary proteins, during intraoral application of CPP-ACP crèmes, might be suggested as reason for the protective effect against caries and erosions. This interactive effect with saliva might be the reason for controversial findings for CPP-ACP in *in situ* and *in vitro* studies [22,24-26].

Therefore, aim of the present *in situ* study was to investigate the potential of a CPP-ACP containing crème to re-harden softened enamel and to evaluate if an intraoral or extraoral application might have an influence on the re-hardening of erosivly softened enamel. It might be suggested, that the re-hardening of previously erosivly softened enamel is enhanced by an intraoral application of CPP-ACP crème due to the higher concentration of calcium and phosphate, and an interaction with saliva. Taking these assumptions into consideration, the hypothesis of the present study was, that CPP-ACP has a higher re-hardening potential when applied intraorally compared with extraoral application and that under *in situ* circumstances the CPP-ACP shows a better re-hardening of softened enamel compared with saliva contact only.

Materials and methods:

Sample preparation:

Two hundred enamel samples were prepared from freshly extracted bovine lower incisors. The teeth were sectioned at the enamel-cementum junction and enamel cylinders were gained from the buccal surface of the crowns by use of a water-cooled diamond trephine mill with an inner diameter of 3 mm. After preparation, the enamel cylinders were embedded in acrylic resin (Paladur, Heraeus Kulzer, Hanau, Germany) and the enamel surface was ground flat and polished using water-cooled carborundum paper (waterproof silicon carbide paper, Stuers, Erkrath, Germany). Before use, baseline surface microhardness (KHN) of all samples was determined and used for stratified allocation of the samples to five groups, which were later correspond to the five experimental series (n = 40; mean KHN in the groups: 339.0 – 339.6). The samples were gamma sterilized (12 kGy, 4 h, Paul Scherrer Institut, Villigen, Switzerland) and stored in tap water until they were used in the study.

Study volunteers:

Ten volunteers (4 male, 6 female, aged 23 - 52 years) were recruited to participate in this study. Exclusion criteria were: patients age of under 18, pregnancy, breastfeeding, patients with known allergies against products to be used in the study or patients with hypo salivation (stimulated: <1.0 ml/min; unstimulated: 0.25 ml/min). Written informed consent after receiving written information concerning the study was obtained from each volunteer. An ethical approval (StV 08/09) was issued by the local ethic committee prior to the recruitment of the volunteers.

Study design:

For each volunteer a custom made intraoral appliance was fabricated to wear four enamel samples in the area buccal of the left and right maxillary second premolar and first molar. Each volunteer had to perform five experimental series (1 run per test solution or control). Between each experimental run, a wash out phase of 7 days was performed. The volunteers had to start

using fluoridated toothpaste (Elmex, GABA, Therwil, Switzerland) 7 days before the experimental run and were asked not to eat or drink two hours before and during the experiment. Directly before each experimental run, four samples were eroded extraorally by immersion in Sprite light (Coca-Cola Company, Atlanta, USA) (2 ml/sample) for 2 min. For each experimental run a new bottle of Sprite light was used. After removing the samples from the Sprite light, the samples were rinsed with tap water to stop the demineralisation process. Now surface microhardness was determined again. After this second measurement the samples were inserted in the intraoral appliance. The volunteers were firstly asked to rinse their mouth with 30 ml of Sprite light and then to insert the appliance. After five minutes intraoral wear, the appliance was removed from the mouth (series 1 and 2) and treated (3 min) extraorally with a fluoride containing mouth rinse (series 1: 250 ppm AmF/SnF_2, Meridol, GABA, Therwil, Switzerland) or a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) containing crème (series 2: GC Tooth Mousse, Leuven, Belgium). From each product 2 ml per sample were applied. After application, the appliances were re-inserted in the participant's mouth. Before re-inserting the appliances, the samples were rinsed with tape water to remove excess material. In series 3 to 5, the appliances remained in the mouth and the volunteer applied 2 ml of the respective product in his mouth (series 3: fluoride containing mouth rinse Meridol; series 4: CPP-ACP containing crème Tooth Mousse) on the enamel samples. The respective amount of the CPP-ACP containing crème (series 4) was applied on the volunteers' finger and he had to apply it on the samples while in series 3 the volunteers were asked to rinse with the respective amount for four samples of the fluoride containing mouth rinse. The volunteers were asked to spit out the applied products after 3 min. In series 5, after erosion the samples remained in the participants mouth and were not treated further (negative control). The appliances were worn intraorally for four hours afterwards. After removing the samples from the appliances, the surface microhardness was again determined. The sequence of series was randomly assigned for each volunteer. A flowchart of the whole study protocol is given in figure 1.

Surface microhardness measurement:

The surface microhardness (KHN) of the enamel samples were determined three times: for stratified allocation (baseline), after immersion of the samples in Sprite light and after completion of the experimental run. For each measurement, four indentations (load weight 50 g, indentation time 20 s) were made on the enamel surface of the samples with a Knoop hardness-measuring device (High Quality Hardness Tester, Buehler, Düsseldorf, Germany) and the mean surface microhardness per sample was calculated. The distance between the indentations was adjusted to minimum 50 µm from each other. To ensure, that no wear of the eroded enamel surface by the intraoral soft tissues during the intraoral wear of the samples occured, the length of the indentations of the second measurement (after erosion) were re-determined after finishing the experimental run and compared with the respective length after erosion. Only a minimal change in indentation length, resulting in a KHN change of less than 4 KHN, was observed. This means, that only a minimal wear of the eroded enamel surface occurs while wearing the samples intraorally. The microhardness measurement was performed by a blinded co-worker, which means that the person conducting the measurement was not aware how the respective samples had been treated.

Statistical analysis:

For statistical analysis, the mean KHN in each series at the three time points of measurement and the mean ΔKHN (final microhardness – microhardness after erosion of the respective samples) were calculated. Mean ΔKHN was calculated to evaluate the re-hardening potential of the respective treatment protocols. Data were statistically analyzed by one-way ANOVA and Bonferroni/Dunn post-hoc test to compare the KHN of the different series within the same time point of measurement and the mean ΔKHN. Furthermore, paired t-tests were performed to compare surface microhardness within the same series at the three time points of measurement. Level of significance was set at 95% and p-values were adjusted for multiple testing (Bonferroni/Dunn).

Results:

Mean surface microhardness (KHN) of the different series (1 – 5) at baseline, after erosion and at final measurement as well as mean ΔKHN are given in table 1.

At the baseline measurement, there was no difference between the surface microhardness of the different series ($p > 0.05$, respectively). Also after erosion, the surface microhardness between the samples of the different series was not statistically significant different.

Extraoral application of the CPP-ACP crème (series 2) resulted in significantly higher surface microhardness (290.2 ± 31.4 KHN) compared to both the extraoral application of the fluoride mouth rinse (series 1; 268.3 ± 25.9 KHN) and the untreated control (270.7 ± 29.0 KHN). Surface microhardness after intraoral application of both CPP-ACP crème (series 4; 277.0 ± 27.4 KHN) and fluoride mouth rinse (series 3; 284.2 ± 28.6 KHN) were not different to the untreated control samples ($p = 0.0368$ and 0.3247, respectively). Furthermore, the final microhardness in series 2 (extraoral application of CPP-ACP crème) was not statistically significantly different compared to that in series 3 (intraoral application of the fluoride mouth rinse) and to that in series 4 (intraoral application of CPP-ACP crème). Irrespective theses findings concerning the final microhardness, no statistically significant difference for the mean ΔKHN (re-hardening) was observed.

Immediately after erosion, surface microhardness in all series was significant lower compared to the respective baseline microharnesses. After respective treatment re-hardening was observed in all series ($p < 0.0004$, respectively). However, baseline values were not achieved ($p < 0.0001$, respectively).

Discussion:

For the present study, bovine enamel has been used to substitute human enamel. Bovine enamel has been used in numerous studies concerning re-hardening and remineralisation of previously due to erosion softened enamel [9,18,27,28]. Advantages of using bovine enamel are

the easy attainability [29] and the similar microstructure and chemical composition compared to human enamel [30-32]. Furthermore, an advantage of bovine teeth is the possibility to gain more then one sample from one tooth and by this being able to reduce differences in baseline properties of the samples.

Evaluation of surface microhardness to evaluate the re-hardening potential of different substrates and to evaluate the susceptibility of eroded enamel, is a common used method [18,28,33,34]. By testing the surface microhardness, no destruction of the sample occurred, so that the same sample can be used for more than one measurement in a row.

The erosive demineralisation of the enamel samples has been performed by immersion the samples in a commercial soft drink, as it has been performed in other studies before [18,35]. Use of a common soft beverage represents the normal *in situ* situation [6,36]. Two minutes exposure time has been used assuming to be representative for a rapid consumption of a soft drink [37] and being more realistic compared with other studies immersion the samples for hours [38,39] or even up to some days [40,41]. In the present study, no cyclic erosive model was used, as the study intended to evaluate possible approaches to re-harden erosivly softened enamel direct after the erosion occurs. Under cycling conditions, with alternating erosion, remineralisation and application of the respective products, it might be imaginable, that the results might be different. Under cycling conditions, an application of the substances is also performed before the erosive challenge. However, it was shown in recent *in vitro* studies [42,43], that even an application of CPP-ACP crème before an erosive attack could not prevent enamel softening or wear due to erosion/abrasion. For the use of fluorides, it has been shown by different studies [12,44] that an application before the erosion can reduce the erosive tooth wear.

Limitation of the present study might be seen in the amounts of products used. For the fluoride containing mouth rinse, the manufacture recommends to use 10 ml for oral application. Dividing these 10 ml by the four samples used in the intraoral appliance, a use of 2.5 ml per sample results. We decided to use the same amount of both kinds of products (CPP-ACP crème

and fluoride mouth rinse), to use 2 ml per sample in the extraoral application and 8 ml for all four samples in the intraoral application. It might be argued that application of 2 ml of the CPP-ACP crème per sample is high, but to stay within the range given for the fluoride mouth rinse, it was assumed that 2 ml were appropriate. If the amounts used for the CPP-ACP crème would have been used for the estimation of the amounts to be used, only very small amounts of fluoride mouth rinse could have been used. If smaller amounts of CPP-ACP crème were used, we suggest that the re-hardening in the series with the CPP-ACP crème might have been even lower.

The hypothesis of the present study has to be rejected as ΔKHN (re-hardening) after intraoral application of the CPP-ACP crème was not statistically significant different compared to the saliva only control group. Furthermore, a higher ΔKHN was observed after extraoral application of the CPP-ACP crème compared with intraoral applications, although this was not significant. Reason for this tendency might be the suggested stimulation of the saliva during the intraoral application of the CPP-ACP crème, resulting in a dilution of the crème while no such dilution occurs during the extraoral application. This dilution results in a lower re-hardening potential during intraoral application. Further reason for the less pronounced re-hardening after intraoral application of the CPP-ACP crème might be contributed to the acquired pellicle formed on the enamel samples during the intraoral wear before application of the crème. It has been shown, that even a short time pellicle may act as a barrier, thus being able to protect the enamel against erosive wear by hampering the contact of the acid with the enamel. It is conceivable that the same mechanism might also hamper the contact of the CPP-ACP containing crème with the enamel. In a resent study by White et al. (2010) [45] a protective effect of caseine phosphopeptide (CPP) against enamel wear due to erosion was found. White et al. (2010) [45] suggested that the adsorbed proteins, modifying or being incorporated in the acquired pellicle, could act as an ion retarding membrane, hampering the approach of H^+ ions to the tooth surface and also inhibit the release of any Ca^{2+} ions which were dissolved during an erosive attack. Furthermore, a buffering effect of the absorbed proteins is suggested, resulting in an increase of

the pH value on the enamel surface while an erosion occurs. The present results for the extraoral application of CPP-ACP crème agree with the findings of Tantbiorj et al. (2008) [20] showing *in vitro* a higher re-hardening capacity of CPP-ACP crème compared with saliva only.

The finding of the present study concerning the re-hardening by intraoral application of a 250 ppm fluoride mouth rinse is in accordance with the findings by Wiegand et al. (2008) [18], showing a significant re-hardening after application, but not achieving the baseline surface microhardness. The increase of surface microhardness is attributed to the incorporation and deposition of fluoride or fluoride compounds into or on the enamel [46-48]. Reason for not achieving the baseline hardness might be a too short application time for sufficient interaction of fluorides with eroded enamel as suggested by previous studies [8,18] or a too low concentration of the applied fluorides, as a study by Attin et al. (1998) [47] showed a proportional relation between fluoride concentration and enamel re-hardening.

Reason for the lower ΔKHN after extraoral application compared with the intraoral application of the fluoride containing mouth rinse might be attributed to the presence of saliva. When fluorides are applied to enamel a calcium fluoride-like precipitate is formed on the enamel [49]. The amount of calcium fluoride-like precipitate depends on the concentration of the applied fluorides, the pH of the applied fluorid, application time and amount of provided calcium ions [16,50,51]. As the concentration, the pH and the application times of the fluoride solutions were similar during intraoral and extraoral application, the difference in ΔKHN has to be attributed to the provided amount of calcium ions. During extraoral application, the calcium ions has to be dissolved from the enamel as no other origin for calcium ions exist, while during intraoral application beside the enamel also the saliva provides calcium ions. So the, even not significant, higher ΔKHN after intraoral application might be attributed to the presence of the saliva.

Also the re-hardening found for the saliva only group of the present study is in agreement with the findings of other studies showing a reduction of the toothbrush susceptibility of eroded enamel with increasing immersion time in saliva [7,52]. The fact that the baseline microhardness

was not achieved within four hours of intraoral wear is in agreement with the findings of an *in vivo* study [28] showing that even after 48 h exposure of eroded enamel to the oral cavity, the pre-study microhardness is not reached.

Within the limitations of the present study we concluded that the intraoral application of both CPP-ACP crème and fluoride containing mouth rinse provides no benefit regarding surface re-hardening of previously erosivly softened enamel compared with untreated control samples. Furthermore, the intraoral application results in no higher ΔKHN (re-hardening) for both the CPP-ACP crème and the fluoride mouth rinse compared with the respective ΔKHN after extroral application. However, irrespective of the mode of application and kind of substances used, the baseline hardness was not achieved.

References:

[1] Petersson GH, Bratthall D. The caries decline: a review of reviews. Eur J Oral Sci 1996;104:436-43.

[2] Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. Monogr Oral Sci 2006;20:44-65.

[3] Wiegand A, Attin T. Influence of fluoride on the prevention of erosive lesions--a review. Oral Health Prev Dent 2003;1:245-53.

[4] Zipkin I, McClure FJ. Salivary citrate and dental erosion; procedure for determining citric acid in saliva; dental erosion and citric acid in saliva. J Dent Res 1949;28:613-26.

[5] Bartlett DW, Coward PY. Comparison of the erosive potential of gastric juice and a carbonated drink in vitro. J Oral Rehabil 2001;28:1045-7.

[6] Lussi A, Jaeggi T, Zero D. The role of diet in the aetiology of dental erosion. Caries Res 2004;38 Suppl 1:34-44.

[7] Attin T, Buchalla W, Gollner M, Hellwig E. Use of variable remineralization periods to improve the abrasion resistance of previously eroded enamel. Caries Res 2000;34:48-52.

[8] Lussi A, Jaeggi T, Gerber C, Megert B. Effect of amine/sodium fluoride rinsing on toothbrush abrasion of softened enamel in situ. Caries Res 2004;38:567-71.

[9] Amaechi BT, Higham SM. In vitro remineralisation of eroded enamel lesions by saliva. J Dent 2001;29:371-6.

[10] Attin T, Knofel S, Buchalla W, Tutuncu R. In situ evaluation of different remineralization periods to decrease brushing abrasion of demineralized enamel. Caries Res 2001;35:216-22.

[11] Lagerweij MD, Buchalla W, Kohnke S, Becker K, Lennon AM, Attin T. Prevention of erosion and abrasion by a high fluoride concentration gel applied at high frequencies. Caries Res 2006;40:148-53.

[12] van Rijkom H, Ruben J, Vieira A, Huysmans MC, Truin GJ, Mulder J. Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride treatment in vitro. Eur J Oral Sci 2003;111:253-7.

[13] Attin T, Deifuss H, Hellwig E. Influence of acidified fluoride gel on abrasion resistance of eroded enamel. Caries Res 1999;33:135-9.

[14] Maia LC, de Souza IP, Cury JA. Effect of a combination of fluoride dentifrice and varnish on enamel surface rehardening and fluoride uptake in vitro. Eur J Oral Sci 2003;111:68-72.

[15] Amaechi BT, Higham SM. Dental erosion: possible approaches to prevention and control. J Dent 2005;33:243-52.

[16] Wegehaupt FJ, Solt B, Sener B, Wiegand A, Schmidlin PR, Attin T. Influence of fluoride concentration and ethanol pre-treatment on the reduction of the acid susceptibility of enamel. Arch Oral Biol 2009;54:823-9.

[17] Lussi A, Jaeggi T, Schaffner M. Prevention and minimally invasive treatment of erosions. Oral Health Prev Dent 2004;2 Suppl 1:321-5.

[18] Wiegand A, Muller I, Schnapp JD, Werner C, Attin T. Impact of fluoride, milk and water rinsing on surface rehardening of acid softened enamel. An in situ study. Am J Dent 2008;21:113-8.

[19] Ranjitkar S, Rodriguez JM, Kaidonis JA, Richards LC, Townsend GC, Bartlett DW. The effect of casein phosphopeptide-amorphous calcium phosphate on erosive enamel and dentine wear by toothbrush abrasion. J Dent 2009;37:250-4.

[20] Tantbirojn D, Huang A, Ericson MD, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. J Dent 2008;36:74-9.

[21] Reynolds EC. Anticariogenic complexes of amorphous calcium phosphate stabilized by casein phosphopeptides: a review. Spec Care Dentist 1998;18:8-16.

[22] Reynolds EC. Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions. J Dent Res 1997;76:1587-95.

[23] Shen P, Cai F, Nowicki A, Vincent J, Reynolds EC. Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. J Dent Res 2001;80:2066-70.

[24] Lennon AM, Pfeffer M, Buchalla W, Becker K, Lennon S, Attin T. Effect of a casein/calcium phosphate-containing tooth cream and fluoride on enamel erosion in vitro. Caries Res 2006;40:154-7.

[25] Willershausen B, Schulz-Dobrick B, Gleissner C. In vitro evaluation of enamel remineralisation by a casein phosphopeptide-amorphous calcium phosphate paste. Oral Health Prev Dent 2009;7:13-21.

[26] Walker GD, Cai F, Shen P, Bailey DL, Yuan Y, Cochrane NJ, Reynolds C, Reynolds EC. Consumption of milk with added casein phosphopeptide-amorphous calcium phosphate remineralizes enamel subsurface lesions in situ. Aust Dent J 2009;54:245-9.

[27] Attin T, Meyer K, Hellwig E, Buchalla W, Lennon AM. Effect of mineral supplements to citric acid on enamel erosion. Arch Oral Biol 2003;48:753-9.

[28] Kim JW, Jang KT, Lee SH, Kim CC, Hahn SH, Garcia-Godoy F. In vivo rehardening of enamel eroded by a cola drink. ASDC J Dent Child 2001;68:122-4, 142.

[29] Oesterle LJ, Shellhart WC, Belanger GK. The use of bovine enamel in bonding studies. Am J Orthod Dentofacial Orthop 1998;114:514-9.

[30] Davidson CL, Boom G, Arends J. Calcium distribution in human and bovine surface enamel. Caries Res 1973;7:349-59.

[31] Gente M, Sondermann U, Lehmann KM. Linear coefficient of thermal expansion for bovine enamel and dentin. Dtsch Zahnarztl Z 1985;40:488-90.

[32] Esser M, Tinschert J, Marx R. Materialkennwerte der Zahnhartsubstanz des Rindes im Vergleich zur humanen Zahnhartsubstanz. Dtsch Zahnarztl Z 1998;53:713-7.

[33] Gedalia I, Dakuar A, Shapira L, Lewinstein I, Goultschin J, Rahamim E. Enamel softening with Coca-Cola and rehardening with milk or saliva. Am J Dent 1991;4:120-2.

[34] Rios D, Honorio HM, Magalhaes AC, Delbem AC, Machado MA, Silva SM, Buzalaf MA. Effect of salivary stimulation on erosion of human and bovine enamel subjected or not to subsequent abrasion: an in situ/ex vivo study. Caries Res 2006;40:218-23.

[35] Hooper S, Hughes J, Parker D, Finke M, Newcombe RG, Addy M, West N. A clinical study in situ to assess the effect of a food approved polymer on the erosion potential of drinks. J Dent 2007;35:541-6.

[36] Johansson AK. On dental erosion and associated factors. Swed Dent J Suppl 2002;1-77.

[37] Meurman JH, Rytomaa I, Kari K, Laakso T, Murtomaa H. Salivary pH and glucose after consuming various beverages, including sugar-containing drinks. Caries Res 1987;21:353-9.

[38] Jain P, Nihill P, Sobkowski J, Agustin MZ. Commercial soft drinks: pH and in vitro dissolution of enamel. Gen Dent 2007;55:150-4; quiz 155, 167-8.

[39] Willershausen B, Schulz-Dobrick B. In vitro study on dental erosion provoked by various beverages using electron probe microanalysis. Eur J Med Res 2004;9:432-8.

[40] Larsen MJ, Nyvad B. Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. Caries Res 1999;33:81-7.

[41] Owens BM, Kitchens M. The erosive potential of soft drinks on enamel surface substrate: an in vitro scanning electron microscopy investigation. J Contemp Dent Pract 2007;8:11-20.

[42] Wang X, Megert B, Hellwig E, Neuhaus KW, Lussi A. Preventing erosion with novel agents. J Dent 2011;39:163-70.

[43] Wegehaupt FJ, Attin T. The Role of Fluoride and Casein Phosphopeptide/Amorphous Calcium Phosphate in the Prevention of Erosive/Abrasive Wear in an in vitro Model Using Hydrochloric Acid. Caries Res 2010;44:358-63.

[44] Hughes JA, West NX, Addy M. The protective effect of fluoride treatments against enamel erosion in vitro. J Oral Rehabil 2004;31:357-63.

[45] White AJ, Gracia LH, Barbour ME. Inhibition of Dental Erosion by Casein and Casein-Derived Proteins. Caries Res 2010;45:13-20.

[46] Wiegand A, Krieger C, Attin R, Hellwig E, Attin T. Fluoride uptake and resistance to further demineralisation of demineralised enamel after application of differently concentrated acidulated sodium fluoride gels. Clin Oral Investig 2005;9:52-7.

[47] Attin T, Zirkel C, Hellwig E. Brushing abrasion of eroded dentin after application of sodium fluoride solutions. Caries Res 1998;32:344-50.

[48] Ganss C, Klimek J, Schaffer U, Spall T. Effectiveness of two fluoridation measures on erosion progression in human enamel and dentine in vitro. Caries Res 2001;35:325-30.

[49] ten Cate JM. Review on fluoride, with special emphasis on calcium fluoride mechanisms in caries prevention. Eur J Oral Sci 1997;105:461-5.

[50] Saxegaard E, Rolla G. Fluoride acquisition on and in human enamel during topical application in vitro. Scand J Dent Res 1988;96:523-35.

[51] Petzold M. The influence of different fluoride compounds and treatment conditions on dental enamel: a descriptive in vitro study of the CaF(2) precipitation and microstructure. Caries Res 2001;35 Suppl 1:45-51.

[52] Jaeggi T, Lussi A. Toothbrush abrasion of erosively altered enamel after intraoral exposure to saliva: an in situ study. Caries Res 1999;33:455-61.

Legends to tables an figures:

Fig. 1: Flowchart of the study protocol.

Tab. 1: Mean (SD) surface microhardness (KHN) of the different series (1-5) at the different time points of measurement and mean ΔKHN (SD) (final microhardness – microhardness after erosion of the respective samples). Values within the same time point of measurement (read

vertically) and within the same series (read horizontally) that are not statistically significantly different are marked with same capital letters. Values for ΔKHN that are not significant different are marked with an asterisk (read vertically last column).

Tables:

		Baseline	**After erosion**	**Final**	**ΔKHN**
Extraoral application	**1** Fluoride mouth rinse	339.6 (15.9) A	249.7 (24.3) B	268.3 (25.9) C	18.6 (21.8) *
	2 CPP-ACP crème	339.3 (15.9) A	263.1 (21.2) B	290.2 (31.4) D	27.1 (24.3) *
Intraoral application	**3** Fluoride mouth rinse	339.0 (15.9) A	259.0 (23.8) B	284.2 C,D (28.7)	25.1 (18.6) *
	4 CPP-ACP crème	339.2 (15.7) A	254.5 (26.6) B	277.0 (27.4) C,D	22.6 (17.0) *
Control	**5**	339.4 (16.1) A	252.2 (27.5) B	270.7 (29.0) C	18.5 (22.2) *

Tab. 1

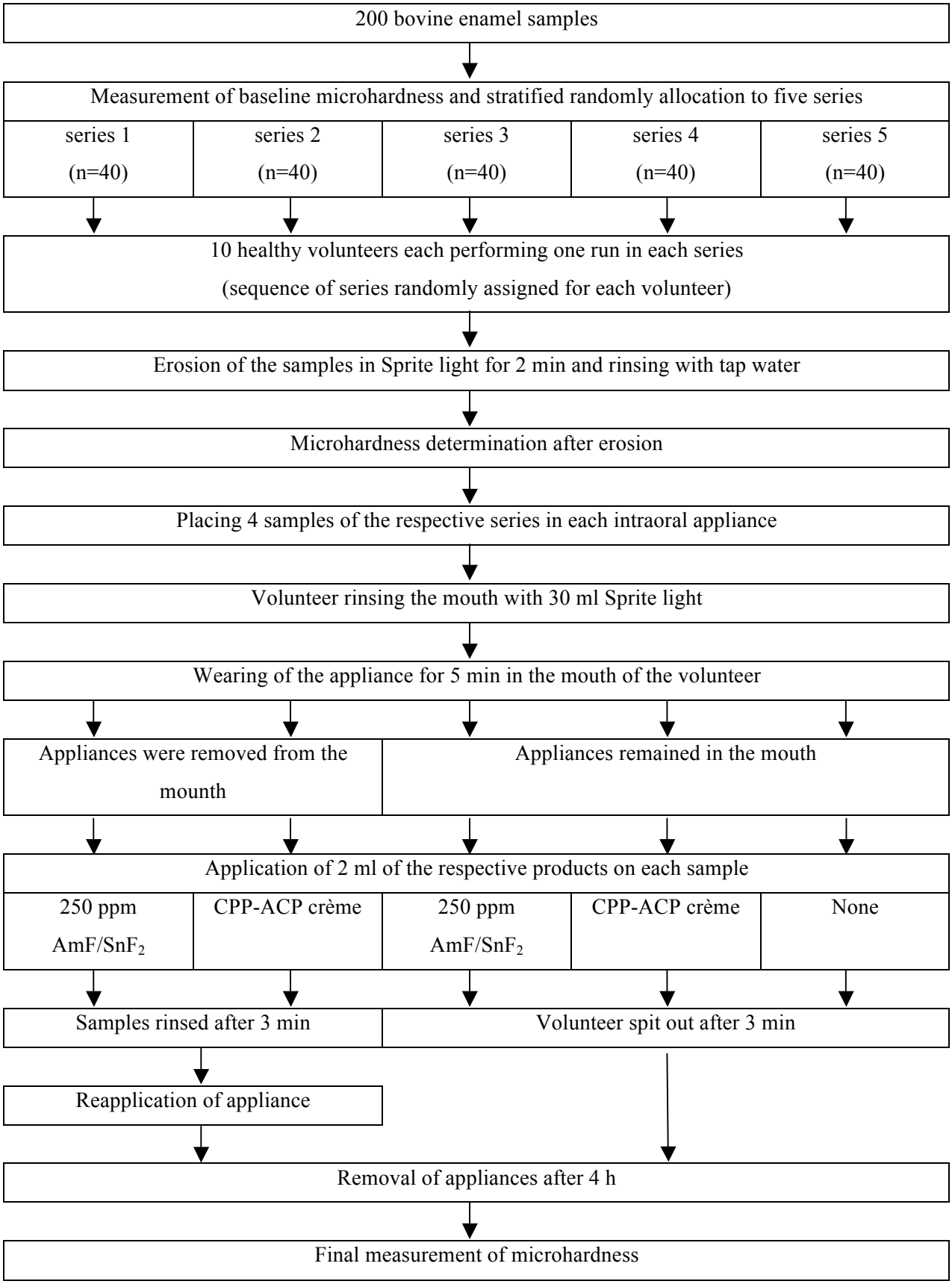

Fig. 1: Flowchart of the study protocol.